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Vitamin D in infectious complications in critically ill patients with or without COVID-19

Alice G Vassiliou[a*], Edison Jahaj[a], Stylianos E Orfanos[a], Ioanna Dimopoulou[a], Anastasia Kotanidou[a*]

[a]First Department of Critical Care Medicine & Pulmonary Services, School of Medicine, National & Kapodistrian University of Athens, "Evangelismos" Hospital, Athens 10676, Greece

Alice G. Vassiliou, alvass75@gmail.com
Edison Jahaj, Edison.jahaj@gmail.com
Stylianos E. Orfanos, sorfanos@med.uoa.gr
Ioanna Dimopoulou, idimo@otenet.gr
Anastasia Kotanidou, akotanid@med.uoa.gr

Corresponding authors

Anastasia Kotanidou, MD, PhD, Professor, First Department of Critical Care Medicine & Pulmonary Services, School of Medicine, National & Kapodistrian University of Athens, "Evangelismos" Hospital, Ipsilantou 45-47, Athens 10676, Greece. akotanid@med.uoa.gr; akotanid@gmail.com

Alice G. Vassiliou, PhD, First Department of Critical Care Medicine & Pulmonary Services, School of Medicine, National & Kapodistrian University of Athens, "Evangelismos" Hospital, Ipsilantou 45-47, Athens 10676, Greece. alvass75@gmail.com

Abstract

25-hydroxyvitamin D [25(OH)D] is an important immunomodulator, whose deficiency may aggravate the incidence and outcome of infectious complications in patients admitted to the intensive care unit. The most recognized extra-skeletal action of vitamin D is the regulation of immune function. Host defense against intracellular pathogens depends upon both innate and adaptive immunity. It has been suggested that vitamin D regulates the pro-inflammatory endothelial response to lipopolysaccharide, rendering it a role in the sepsis cascade. Recent studies have indicated that vitamin D deficiency may be associated with worse outcomes in patients with coronavirus disease 2019 (COVID-19), such as more severe disease and higher mortality rates. To this end, clinical trials with vitamin D supplementation are being carried out in an effort to improve COVID-19 outcomes. In this review, we will discuss the role of vitamin D in the immune response, and more specifically its effect on immune cells. Subsequently, we will provide an overview of the studies that have investigated the predictive value of vitamin D in critical illness outcomes, and its therapeutic value as a supplement in critically ill patients. Finally, the emerging role of vitamin D deficiency in COVID-19 infection risk, and worse outcomes will be discussed.

Key Words: Vitamin D; Critical illness; Infections; Outcomes; COVID-19

Introduction

Vitamin D was primarily recognized for its role in calcium homeostasis, whose deficiency caused rickets [1]. In the recent years, vitamin D has been found to play an important role in modulating immune cells, and inhibiting the inflammatory response [2]. Vitamin D is implicated in the regulation of over 2000 genes, is known to respond to infection, plays a role in antimicrobial peptide production, and triggers innate immunity. The overall result of vitamin D deficiency is the alteration of key immune response biological processes, such as gene expression, cytokine production, metabolism and cell function [3, 4].

Studies have revealed a high prevalence of vitamin D deficiency in critically-ill patients, and that vitamin D deficiency might be associated with worse outcomes in patients with coronavirus disease 2019 (COVID-19), such as more severe disease and higher mortality rates. Many risk factors have been recognized for decreased vitamin D levels, including age, latitude, the use of sunscreen, limited sun exposure, non-White ethnicity, obesity, low dietary intake of vitamin D, and malabsorption syndromes. However, the low vitamin D levels seen in critically-ill patients may be a result of many factors, including drug interactions, irregular gastrointestinal function and the result of fluid resuscitation [5].

This review will discuss the findings associated with vitamin D and the risk and severity of complications from infections in the intensive care unit (ICU), including COVID-19.

It should be noted, however, that all of the studies mentioned measured the circulating form of vitamin D, 25(OH)D. Data from studies suggest that its active form, $1,25(OH)_2D$, is responsible for almost every biological function, including the antimicrobial and immunomodulatory actions of vitamin D discussed [6]. Emerging evidence suggests that other related molecules may contribute to the individual's vitamin D status (e.g., vitamin D binding protein, bioavailable and free 25(OH)D, and $1,25(OH)_2D$). However, the measurement of these molecules is complex, and it still has not been decided whether their measurement is merited in new research studies. 25(OH)D is currently the best marker for overall vitamin D status, and hence remains the most commonly measured biomarker in clinical medicine. Herein, we will use the term vitamin D.

Vitamin D production and metabolism

Vitamin D3 (cholecalciferol) is made in the skin from 7-dehydrocholesterol when exposed to UVB light. Vitamin D2 (ergocalciferol) is derived from the plant sterol ergosterol. Vitamin D is metabolized first to 25-hydroxyvitamin D (25(OH)D), then to the active metabolite 1,25-

dihydroxyvitamin D ($1,25(OH)_2D$). The liver has been established as the major source of 25(OH)D production from vitamin D, while the kidney is the major source of circulating levels of $1,25(OH)_2D$ [7]. All genomic actions of $1,25(OH)_2D$ are mediated by the vitamin D receptor (VDR). VDR is a transcription factor that exists in nearly every tissue, and member of the steroid hormone nuclear receptor family. VDR binds to DNA sites termed vitamin D response elements (VDREs). There are thousands of these binding sites throughout the genome regulating hundreds of genes in a cell-specific fashion. Figure 1 illustrates vitamin D metabolism and signaling. Serum total 25(OH)D, the sum of 25(OH)D2 and 25(OH)D3, is the best reflection of vitamin D status.

Vitamin D and immunity

Vitamin D deficiency has been shown to be associated with worse outcomes of infectious complications, especially in patients admitted to the ICU [8]. The most recognized extra-skeletal action of vitamin D is the regulation of immune function [9]. Vitamin D is an important link between toll-like receptor (TLR) activation, leukocyte accumulation, local inflammation, and antibacterial responses in innate immunity [10-12]. TLRs are essential in innate and adaptive immune responses. Macrophages recognize lipopolysaccharide through TLRs, leading to a series of events, which result in the production of peptides with potent bactericidal activity, namely cathelicidin and β-defensin. These peptides co-localize with ingested microbes, within phagosomes, disrupting their cell membranes [13]. Besides antimicrobial activity, these peptides have antiviral activity, and can also inactivate the influenza virus [14]. Vitamin D deficiency has been shown to be associated with reduced TLR expression levels; therefore 25(OH)D ultimately modulates the expression of cathelicidin and β-defensin, which may enhance endothelial barrier function [15].

Experimental studies have shown that vitamin D affects numerous immune cells. It inhibits B cell proliferation and differentiation, blocks immunoglobulin secretion [16], and suppresses T cell proliferation [17]. In response to microbial pathogens, $CD4^+$ T cells differentiate into T-helper (Th)1 or Th2 cells; vitamin D causes a shift from a Th1 to a Th2 phenotype [18, 19]. The Th1 subset produces interferon-gamma and lymphotoxin, and plays an important part in the protection against intracellular bacteria and protozoa; additionally, it has been associated with autoimmune pathologies. Th2 helper cells lead to a humoral immune response, by producing interleukin (IL)-4, IL-5, and IL-13, usually against extracellular parasites. Hence, differentiation of CD^{4+} T cells into either Th1 or Th2 cells will determine the outcome of an immune response. Differentiation is

primarily directed by cytokines; IL-12 induces the development of Th1 cells, whereas Th2 cells develop in response to IL-4 [18]. Furthermore, vitamin D affects T cell maturation, inhibits the proliferation of Th17 cells, and facilitates the induction of regulatory T cells [20-23]. All these vitamin D effects have been proposed to result in decreased production of inflammatory cytokines, such as IL-1, IL-6, IL-8, IL-12, IL-17, IL-21 and tumor necrosis factor alpha (TNF-α) [24], and increased production of anti-inflammatory cytokines, such as IL-10 [25, 26].

Vitamin D also has effects on macrophages, monocytes, and dendritic cells (DCs). It modulates the phagocytic activity of macrophages, and inhibits the production of the inflammatory cytokines IL-1, IL-6, IL-8, IL-12, and TNF-α from monocytes [27, 28]. It additionally inhibits differentiation and maturation of the antigen-presenting DCs, resulting in decreased expression of major histocompatibility complex class II molecules, and stimulation of IL-12 [29].

Figure 2 summarizes the established effects of vitamin D on immune cells.

With the regards to the effect of vitamin D on natural killer (NK) cell functions, contradictory results have been reported from *in vitro* studies [30-32]; whether vitamin D induces or inhibits NK cell function *in vivo* is still unclear [33]. Low circulating NK cell counts have been associated with more severe phenotypes of common variable immunodeficiency, implying a protective role of NK cells against severe bacterial infections, when the adaptive immune response is not the most appropriate [34].

In vitro data have shown that, in addition to modulating innate immune cells, vitamin D also induces immune tolerance. Data from animal and human studies with vitamin D supplementation, have demonstrated the beneficial effects of vitamin D on immune function *in vivo*, especially on autoimmunity [35-37].

Vitamin D deficiency has been linked to a wide range of metabolic disorders, including malignant, cardiovascular, infectious, neuromuscular, and autoimmune diseases. Local synthesis of vitamin D has been shown to mediate T cell responses, whereas patients with diseases that affect vitamin D metabolism, such as chronic renal failure and rickets, have impaired activity of NK cells [36]. These immunomodulating effects of vitamin D may be responsible for the epidemiological associations between vitamin D status and autoimmune and inflammatory diseases [38].

Vitamin D in critically-ill patients

The predictive value of vitamin D in the ICU has been the focus of many studies. However, controversial results have been reported on its predictive and therapeutic value. Vitamin D

deficient patients have been shown to have higher infection rates, although not significant [39, 40], whereas no difference was observed between patients with bacteremia, pneumonia, urinary tract infection, skin-soft tissue infection, or intra-abdominal infection [39]. Vitamin D deficiency has been associated with increased risk of blood culture positivity compared to vitamin D sufficiency [41]. In critically-ill septic patients, extremely low (< 7 ng/mL) vitamin D levels on ICU admission may contribute to poor ICU outcomes, including mortality and higher infection rates [42]. Our group showed that severely low vitamin D levels on ICU admission were associated with a higher rate of respiratory tract infections in initially non-septic patients [43]. The association of vitamin D deficiency with worse outcomes in the critically-ill, including sepsis, mortality, duration of mechanical ventilation, and length of stay, has been demonstrated in a plethora of studies [5, 8, 39-41, 44-48]. Very recently, it was shown that vitamin D deficient critically-ill patients had more complications relating to pneumonia severity, such as sepsis and acute respiratory distress syndrome (ARDS), and worse outcomes [49]. A meta-analysis of randomized controlled trials (RCTs) demonstrated that vitamin D supplementation may prevent acute respiratory tract infections, especially in severely vitamin D deficient patients not receiving bolus doses [50]. Other reports, however, have not been able to demonstrate an association between vitamin D deficiency and poor ICU outcomes [51-53], or risk of hospital-acquired infections [54].

It has been proposed that the higher mortality rates, seen in vitamin D deficient and insufficient critically-ill patients, may be a result of glucose and calcium metabolism abnormalities and/or immune and endothelial cell dysfunction [44]. There is still dispute as to whether vitamin D reduces inflammation or whether inflammation decreases vitamin D levels.

Due to the confounding associations and interactions, it is challenging to prove cause and effect on the outcomes of the critically-ill patients. Nonetheless, results from studies showing associations of vitamin D deficiency with worse outcomes, prompted clinical trials to investigate whether vitamin D supplement therapy improves outcomes in critically-ill patients. The largest RCT, VITdAL-ICU, which tested high dose cholecalciferol supplementation in a mixed population of critically-ill patients with vitamin D deficiency, showed that vitamin D3 was not able to reduce ICU or hospital length of stay, in-hospital mortality, or 6-month mortality [55]. In critically-ill patients, although studies have revealed a high prevalence of vitamin D deficiency, vitamin D supplement therapy has yet to be proven beneficial on outcomes.

Vitamin D in COVID-19

COVID-19 usually presents with pneumonia, severe ARDS, myocarditis, microvascular thrombosis and/or cytokine storm, all of which involve underlying inflammation. Reduced levels have been associated with an increase in inflammatory cytokines and an increased risk of developing pneumonia and viral upper respiratory tract infections [56], including community-acquired pneumonia, the most common medical cause for hospital admissions [57, 58]. Low plasma vitamin D levels have been shown to constitute an independent risk factor for COVID-19 infection and hospitalization [59], while severe acute respiratory syndrome coronavirus 2 (SARS-CoV2)-positive patients have reduced vitamin D levels compared to negative patients [60, 61]. Moreover, vitamin D deficiency increased the risk of developing COVID-19 [62]. In a Northern Italy hospital, however, the averaged vitamin D levels were similar in SARS-CoV2-positive and negative patients [63]. This was also observed in a Brazilian study, where the authors concluded that in their specific population, clinical, environmental, socioeconomic, and cultural factors had a greater influence, rather than vitamin D levels, in determining susceptibility to COVID-19 infection [64]. In an Arab Gulf country, vitamin D deficiency was not associated with SARS-CoV2 infection, but the authors suggested that it may increase the mortality risk in severely deficient cases [65]. A study in hospitalized COVID-19 patients showed that higher vitamin D levels were associated with less extent of lung involvement, and with a significant decrease in the risk of mortality, instigating the authors to suggest that vitamin D might be protective against the development of severe disease, once infected [66]. Vitamin D deficiency has also been associated with the progression and severity of COVID-19, as documented by the need for invasive mechanical ventilation and/or mortality [67-74]. Thus, it has been proposed that diagnosis of vitamin D deficiency could aid in identifying patients prone to developing severe COVID-19, defined as presence of ARDS and/or need for mechanical ventilation, ICU *vs* ward admission, and lower survival probability. Vitamin D levels were lower in hospitalized COVID-19 patients compared to healthy controls, however, no relationship could be established between vitamin D concentrations and the severity of the disease or its clinical course [75, 76]. No significant association between vitamin D levels and severity, mortality, mechanical ventilation, ICU admission, and the development of thromboembolism was observed in COVID-19 patients [77, 78]. Lower vitamin D levels were associated with SARS-CoV2 infection and mortality in the Indian population [79], and among Asian countries [80]. In a Tehran referral hospital, vitamin D levels were not associated with mortality, however the authors concluded that in severe COVID-

19, vitamin D deficiency seemed to affect the course of the disease and mortality, especially in comorbid and older patients [81]. In a Mendelian randomization study, evidence supporting an association between 25(OH)D levels and COVID-19 susceptibility, severity, or hospitalization was not observed [82].

In a large meta-analysis, vitamin D deficiency was not associated with a higher chance of infection by SARS-CoV2; nevertheless, severe cases of COVID-19 were more vitamin D deficient compared to mild cases. Moreover, vitamin D insufficiency increased risk of infection, hospitalization and mortality rates, and a positive association was observed between vitamin D deficiency and COVID-19 severity [83-85]. Two large meta-analyses assessed the association between vitamin D deficiency and COVID-19 incidence, complications, and mortality, in 46 and 43 countries, respectively. The results of the analyses suggested an association between vitamin D deficiency and SARS-CoV2 infection risk, COVID-19 disease severity, and mortality risk [86, 87]. Other systematic reviews and meta-analyses have indicated that low vitamin D levels might be associated with an increased risk of COVID-19 infection [88, 89], and/or COVID-19 severity, and mortality [90]. With regard to infection, the authors suggested caution in interpreting the results, due to inherent study limitations, while for ICU admission, hospitalization, and pulmonary involvement, they concluded that the evidence is inconsistent and insufficient [90]. A strong correlation between severe vitamin D deficiency prevalence and COVID-19 mortality rate in Europe was established, following the analysis of data sets from 10 countries [91]. In elder patients with low vitamin D levels, cytokine storm markers were elevated, and those patients presented with hypoxia requiring non-invasive ventilatory support [92]. On the contrary, other studies do not support a relationship between pre-hospitalization vitamin D status and COVID-19 clinical outcomes [93].

Vitamin D in critically-ill COVID-19 patients

In critically-ill COVID-19 patients there are far less studies. One study demonstrated that vitamin D levels were very low, and that the corresponding inflammatory response and fatality rate were higher in critically-ill patients compared to asymptomatic carriers [94]. The lowest vitamin C and D levels were found in critically-ill patients. However, a study showed that it was older age and low vitamin C levels that were co-dependent risk factors for mortality, and not vitamin D levels [95]. In a respiratory intermediate care unit, patients with 25(OH) levels below 10 ng/mL had a 50% mortality probability, following a 10-day hospitalization period [96]. Our group was able to

demonstrate that in a small cohort of critically-ill patients, lower ICU admission vitamin D levels (<15.2 ng/mL) were associated with an increased 28-day ICU mortality risk [97]. In another study, we found that in critically and non-critically-ill COVID-19 patients, vitamin D deficient COVID-19 patients (< 20 ng/mL) had a decreased number of NK cells [98]. In COVID-19 patients, reduced numbers of NK cells and exhaustion have been linked to the progression and severity of COVID-19 [99-101]. In severe disease, NK cells have been shown reduced but strongly activated; the authors suggested that it was their activation that correlated with the development of severe disease [102]. On the contrary, no difference was observed in the vitamin D levels of those hospitalized and those admitted to the ICU, and furthermore, among the ICU patients, there were no significant differences in ICU clinical outcomes between patients with low and normal vitamin D levels [103]. Similar results were observed in another study, in which 96% of critically ill COVID-19 ARDS patients exhibited vitamin D deficiency; however, the low levels of 25(OH)D were not related to changes in clinical course, whereas the low levels of 1,25(OH)D were associated with prolonged mechanical ventilation [104].

Table 1 summarizes the findings of the vitamin D studies in critically ill COVID-19 patients.

Vitamin D supplementation studies in COVID-19

Due to the above, clinical trials with vitamin D supplementation are underway in COVID-19 patients in an effort to improve outcomes. In the frail elderly with less severe COVID-19, a single oral bolus vitamin D3 dose of 50,000 IU per month, or 80,000-100,000 IU every 2-3 months, during, or just before COVID-19 infection, was associated with a better survival rate [105, 106]. Very recently, a cross-sectional multicenter observational study showed that a high dose cholecalciferol booster therapy (approximately ≥ 280,000 IU in a time period of up to 7 weeks) was associated with a reduced risk of COVID-19 mortality [107]. A retrospective study suggested a potential benefit of cholecalciferol (400,000 IU bolus oral cholecalciferol, 200,000 IU administered in two consecutive days) in comorbid COVID-19 patients [108]. In outpatients, 10,000 IU of vitamin D3, for 14 days resulted in fewer symptoms compared to the control group [109]. On the other hand, administration of 200,000 IU vitamin D3 as a loading dose and 10,000 IU daily thereafter via enteral feeding did not impact the biologically active metabolite 1,25(OH)D, prompting the authors to suggest that both forms should be included in monitoring vitamin D status, and future interventional studies should target the usefulness of calcitriol administration in COVID-19 patients [104]. A systematic review and meta-analysis on vitamin D supplementation

and clinical outcomes in COVID-19, including 10 observational studies and 3 RCTs, concluded that vitamin D supplementation might be associated with improved clinical outcomes, especially when administered after the diagnosis of COVID-19 [110]. The results of the pragmatic trial underway of Wang *et al*. of parallel testing vitamin D3 supplementation for early treatment and post-exposure prophylaxis of COVID-19 are being eagerly awaited [111]. Issues regarding the appropriate dose, duration, and mode of administration of vitamin D remain unanswered and need further research. Ongoing vitamin D supplementation trials have been extensively reported in [112].

It becomes evident, that further studies are warranted to evaluate the effect of vitamin D levels on the prognosis of COVID-19 patients, and the impact of vitamin D supplementation on clinical severity.

Conclusion

Vitamin D has important immunomodulatory effects. The high prevalence of vitamin D deficiency in critically ill patients, including those with COVID-19, has been shown to be associated with worse outcomes of infectious complications. The need for more observational studies with COVID-19 patients to assess the role of vitamin D in relation to risk, disease severity, and outcomes is evident. More randomized clinical trials with larger populations are also required to evaluate the efficacy of supplementation, in both a therapeutic and preventive context.

Abbreviation list

1,25(OH)2D: 1,25-hydroxyvitamin D; 25(OH)D: 25-hydroxyvitamin D; ARDS: Acute respiratory distress syndrome; COVID-19: Coronavirus disease 2019; DC: Dendritic cell; ICU: Intensive care unit; Ig: Immunoglobulin; Il: Interleukin; MHC: Major histocompatibility complex; NK: Natural killer; RCT: Randomized controlled trial; SARS-CoV2: Severe acute respiratory syndrome coronavirus 2; Th: T-helper; TLR: Toll-like receptor; TNF-α: Tumor necrosis factor α; VDR: Vitamin D receptor; VDRE: Vitamin D response element

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Author contributions

Vassiliou AG, Jahaj E, Orfanos SE, Dimopoulou I and Kotanidou A drafted the manuscript; Vassiliou AG, Dimopoulou I and Kotanidou A performed the final editing.

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Table 1: Vitamin D studies in critically ill COVID-19 patients.

Cut-off for vitamin D	Patient cohort	Outcome/Findings	Reference
<30 ng/mL	154 patients: 91 asymptomatic COVID-19 patients and 63 severely ill patients requiring ICU admission	Vitamin D level is markedly low in severe COVID-19 patients	[94]
<10 ng/mL	42 patients with acute respiratory failure due to COVID-19, treated in Respiratory Intermediate Care Unit (RICU)	Patients with severe vitamin D deficiency had a 50% mortality probability	[96]
<15.2 ng/mL	30 critically ill COVID-19 patients	The low vitamin D group had an increased risk of 28-day ICU mortality	[97]
<50 nmol/L	50 patients admitted to the ICU	No significant differences in ICU clinical outcomes (invasive and non-invasive mechanical ventilation, acute kidney injury and mechanical ventilation and hospital days) between patients with low and normal vitamin D levels	[103]
<30 ng/mL	26 critically ill COVID-19 ARDS patients	96% of critically ill COVID-19 ARDS patients suffered from vitamin D deficiency. Low vitamin D levels were not related to changes in clinical course	[104]

Table 2: Vitamin D supplementation studies.

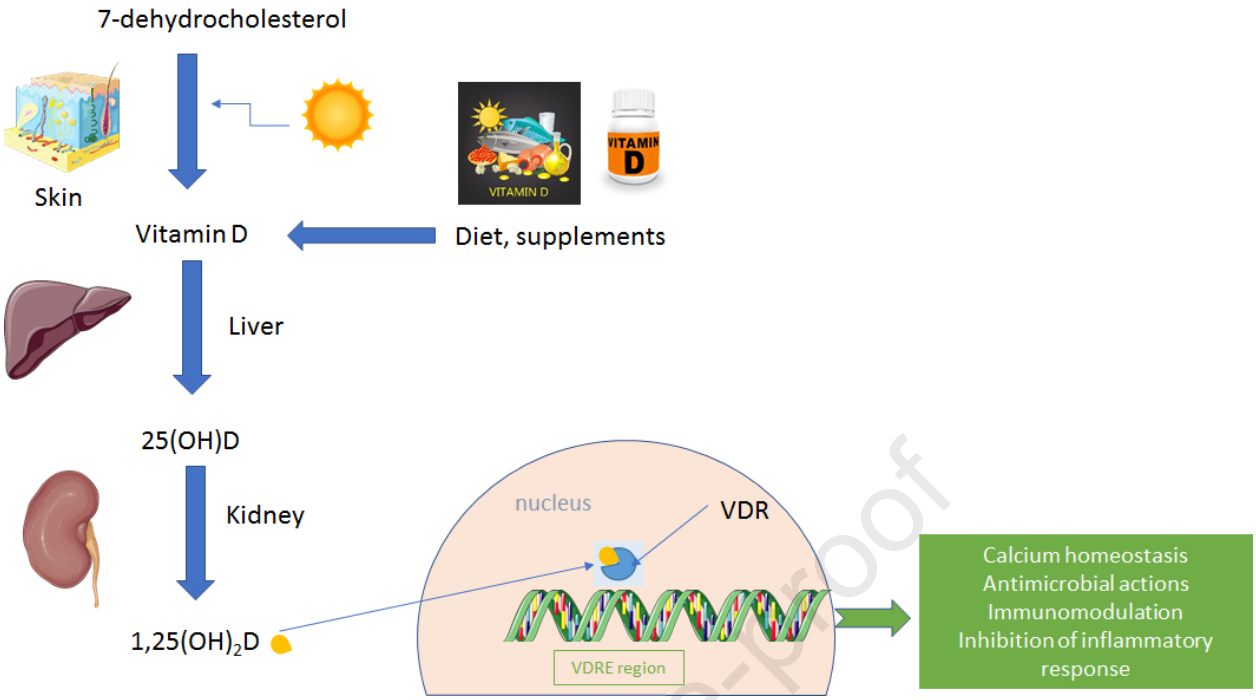
Vitamin D3 dose/Intervention	**Patient cohort, N**	**Outcome/Findings**	**Reference**
Group 1: an oral bolus of 80,000 IU either in the week following the suspicion or diagnosis of COVID-19, or during the previous month. Group 2: None, comparator	Intervention group: N= 57; Comparator: N= 9	Association with a better survival rate	[105]
Group 1: a single oral bolus dose of 50,000 IU per month or 80,000-100,000 IU every 2-3 months. Group 2: an oral supplement of 80,000 IU within a few hours of the diagnosis of COVID-19. Group 3: None, comparator	77 patients; Group 1: N= 29; Group 2: N= 16; Group 3: N= 32	Regular bolus vitamin D supplementation was associated with less severe COVID-19 and better survival in frail elderly	[106]
40,000 IU one-off dose or up to	444 COVID-19 patients. Cholecalciferol booster therapy: N= 151	Cholecalciferol booster therapy was associated	[107]

350,000 IU (booster therapy)		with a reduced risk of COVID-19 mortality	
400,000 IU bolus oral cholecalciferol (200,000 IU administered in two consecutive days)	Bolus: N= 36; Best available treatment: N= 55	Beneficial effect of cholecalciferol on outcome (transfer to ICU or death)	[108]
200,000 IU as a loading dose and 10,000 IU daily via enteral feeding	N= 26 critically ill COVID-19 ARDS patients	Supplementation did not impact the biologically active metabolite 1,25(OH)D	[104]
Randomization to either vitamin D3 (loading dose, then 3200 IU/day) or placebo in a 1:1 ratio	2700	Recruiting. VIVID trial	[111]
10,000 IU for 14 days	42 outpatients; Vitamin D: N=22; Control: N= 20	On the 14th day, the supplemented group presented fewer symptoms compared to the control group	[109]

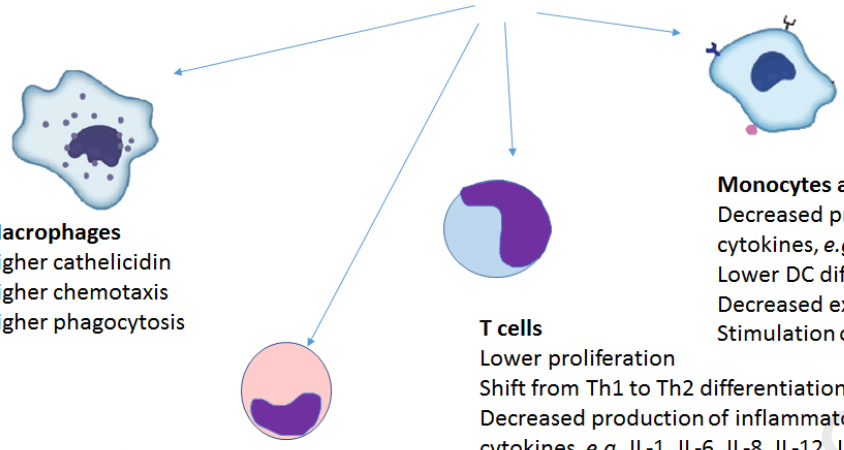
Figure Legends

Figure 1. Vitamin D metabolism and signaling. Vitamin D3 (cholecalciferol) is made in the skin from 7-dehydrocholesterol when exposed to UVB light. Vitamin D2 (ergocalciferol) is derived from the plant sterol ergosterol. Vitamin D is metabolized first in the liver to 25-hydroxyvitamin D (25(OH)D, calcidiol), then in the kidneys to the active metabolite 1,25-dihydroxyvitamin D ($1,25(OH)_2D$, calcitriol). $1,25(OH)_2D$ enters the cell nucleus, where it binds to the vitamin D receptor (VDR). VDR binds to DNA sites termed vitamin D response elements (VDREs). The end result is gene expression regulation, resulting in the various functions of vitamin D, such as maintenance of calcium homeostasis, antimicrobial and immunomodulatory actions, and the inhibition of the inflammatory response.

Figure 2. The effects of vitamin D on immune cells. Vitamin D has effects on various immune cells, including B and T cells, macrophages, monocytes, and dendritic cells. The final result is regulation of the immune response. DC: Dendritic cell; Ig: Immunoglobulin; IL: Interleukin; MHC: Major histocompatibility complex; Th: T-helper cell; TNF-α: Tumor necrosis factor-α.



VITAMIN D EFFECT ON IMMUNE CELLS



Macrophages
Higher cathelicidin
Higher chemotaxis
Higher phagocytosis

B cells
Lower differentiation
Lower proliferation
Lower IgG and IgM production

T cells
Lower proliferation
Shift from Th1 to Th2 differentiation
Decreased production of inflammatory cytokines, *e.g.* IL-1, IL-6, IL-8, IL-12, IL-17, IL-21 and TNF-α
Increased production of anti-inflammatory cytokines, IL-10
Increased T cell maturation
Induction of regulatory T cells
Skewing away from Th17 inflammatory phenotype

Monocytes and DCs
Decreased production of inflammatory cytokines, *e.g.* IL-1, IL-6, IL-8, IL-12, TNF-α
Lower DC differentiation and maturation
Decreased expression of MHC class II molecules
Stimulation of IL-12